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ORIGINAL ARTICLE

Facile synthesis of indole-pyrimidine hybrids and evaluation of their anticancer and antimicrobial activity

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Abstract The paper describes the facile synthesis of new *N*-cyclopropyl-1-methyl-1*H*-indole-2-carboxamide derivatives bearing substituted 2-amino pyrimidine moiety at position-3 of the indole ring. All the intermediate and title compounds were characterized adeptly by ^{1}H NMR, ^{13}C NMR, ESI–MS and elemental analyses. These compounds were evaluated for their *in vitro* anticancer activity against HeLa, HepG2 and MCF-7 cells. Three among 22 molecules, showed more than 70% growth inhibition against all three tested cancer cells. The nature of the substituent group on the pyrimidine ring (R^2) affected significantly the anti-proliferative activity of the molecules. The anti-microbial evaluation of the title molecules revealed the significance of fluoro/chloro groups (R^2) in enhancing their inhibition activity. Eight molecules which contain fluoro/chloro groups showed potent anti-microbial activity. In addition, the active molecules displayed negligible toxicity to benign Vero cells.

1. Introduction

The term 'cancer' encompasses an extensive range of malignancies and is currently the leading cause of death in developed countries and comes second in developing countries [1]. Affliction due to cancer is increasing exponentially owing to its innate characteristics *viz*. uncontrolled proliferation, invasion and metastasis. Its treatment remains an important and challenging therapeutic task in the field of medicinal chemistry since majority of the tumours are either not completely curable or fail to respond to chemotherapy. Although infections by drug-sensitive strains can be successfully cured, the emergence of

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drug resistance has prompted the discovery and development of new, more active and less toxic anti-tumour drugs. Heterocyclic motifs are of immense biological interest due to their occurrence in many natural products and their varied physicochemical properties. Various classes of such heterocycles with diverse chemical structures are used as chemotherapeutic agents or are under clinical trials. Amidst all of them, we have chosen the indole unit as a lead molecule because the 3-substituted indoles, in particular, have proven to be versatile intermediates for the synthesis of a wide range of bioactive drugs [2–4]. Further, literature survey reveals that a combination of two or more active structural moieties can possibly augment the bioactivity. A variety of *N*-based heterocycles *viz*. pyridine [5], quinolone [6], quinazoline [7], etc. have been integrated with the indole nucleus to get potent molecules. Leading amongst them is the pyrimidine ring system which has a distinguished account starting from its discovery phase as a constituent of nucleic acids to their present-day use in chemotherapy [8]. The pyrimidine ring is found in vitamins like thiamine, riboflavin and folic acid [9]. One of the early antimetabolite 5-fluorouracil, a pyrimidine derivative, is used as an anti-neoplastic agent [9]. Over the years, pyrimidine ring systems have been widely explored for their diverse range of biological activities [10–15]. In addition, many pyrimidine analogues have been developed as chemotherapeutic agents [16], anti-leukaemic drugs [17], calcium-sensing receptor antagonists [18], etc. It will be interesting to see indole and pyrimidine moieties, which are considered to be potent pharmacophores on their own, to be incorporated into a single structural entity for better efficacy in terms of their pharmacological activity.

As reviewed from earlier literature reports, efforts have been made to assimilate indole and pyrimidine analogues to afford novel chemical entities with appreciable biological activity. Most of them either had a carbonyl group or its analogue thiocarbonyl at position-2 of the pyrimidine heterocycle and an unsubstituted N–H group on the indole as well as on the pyrimidine unit. Such compounds were found to be potent as antitumour [19,20], antimicrobial [21,22] and antioxidant [23] agents. Moreover, meridianins *viz*. marine indole alkaloids (comprising of indole units bearing 2-amino pyrimidine ring) from tunicate *Aplidium meridianum* have also been isolated and their total synthesis was carried out (Fig. 1) [24,25]. These natural products not only exhibit cytotoxicity but also inhibit CDKs, GSK-3, PKA and other protein kinases [26]. Further, some derivatives of meridianins have shown excellent inhibition against MCF-7 and HeLa cells [27]. Also, pyrimidine heterocycles possessing a free amine group at position-2 of the ring have acquired a unique place in medicinal chemistry. In fact, the 2-amino group is a common structural feature in some well-established pyrimidine drugs [28]. Also, introduction of amide moiety at the position-2 of indole enhanced the antiproliferative effect by HDAC (histone deacetylase) inhibition activity as compared to its substitution at other positions of the indole ring [29]. Based on these facts, we envisaged them to be effective substitutes for esters in improving the potency of synthesized compounds. In the case of amides, the cycloalkyl [30] moiety was chosen as it is considered to be an isostere of olefin [31]. Besides, a cyclopropyl group could be used in SAR studies in place of a geminal-dimethyl group or a geminal-difluoro group. A cyclopropyl group may also provide metabolic stability because of its inherent bond angle leading to enhanced activity of the synthesized molecules [31] prompting us to incorporate a cyclopropyl amide moiety at position-2 of the indole ring. Hence, in the ongoing search for potent chemical entities and with reference to the aforesaid facts, we aimed to synthesize molecules comprising of these two pharmacophores in a single molecular framework. In view of this, a new library of indole-pyrimidine hybrids were prepared by the cyclization of indole-3-chalcones. In addition, the bioisosteric replacement of hydrogen by fluorine [32] at position-5 and ester by amide linkage at position-2 of indole nucleus [29,30] was also carried out for improving the biological potency of the synthesized molecules. All the title compounds were investigated for their anti-cancer activity over three human cancer cell lines, by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In addition, we have tested these molecules for their antimicrobial activity as well against a few strains of bacteria and fungi.

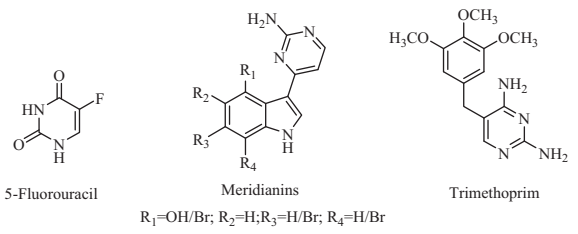

Figure 1 Structures of some indole and pyrimidine based bioactive molecules.

2. Experimental

2.1. Materials and methods

All the chemicals used in the present work were procured from Sigma Aldrich (Germany) and Spectrochem Chemicals Pvt. Ltd. All the solvents used are of analytical grade. They were purchased, distilled and dried before their usage. The progress of the reaction was monitored by thin layer chromatography, performed on a Silica gel 60 F254 coated aluminium sheet. Melting points were determined on open capillaries using a Stuart SMP3 (BIBBY STERLIN Ltd. UK) apparatus and were uncorrected. IR spectra were recorded on a Bruker Alpha Eco-ATR spectrometer as neat sample. The ^{1}H NMR spectra of the intermediates and final compounds were recorded with a Bruker 400 MHz NMR spectrometer using TMS as internal reference and DMSO-d_6 as the solvent. The ^{13}C NMR spectra of the compounds were recorded using a Bruker 100 MHz NMR spectrometer. Elemental analyses were performed on a Flash EA-1112 CHNS analyzer (Thermo Electron Corporation). Mass spectra were recorded on Agilent Triple quad LC–MS model: 0430, using methanol as the solvent. All the reactions, except for the hydrolysis step, were performed under strict inert conditions.

2.2. Synthesis

2.2.1. Procedure for synthesis of ethyl 1-methyl-1H-indole-2-carboxylate (*5a*)

The ethyl 1*H*-indole-2-carboxylate intermediate **4a** (6 g, 31.71 mmol) was suspended in anhydrous dimethyl formamide

(DMF) (60 mL) in a dry round bottomed flask to which potassium carbonate (8.7 g, 52.85 mmol) was added at 0 °C. *Tetra-n*-butylammonium bromide (*n*-TBAB) was added in catalytic amount to this mixture. It was then stirred at room temperature for 30 min following which methyl iodide (6 mL, 95.13 mmol) was added. The reaction mass was then kept for stirring for 12 h. The progress of the reaction was monitored using TLC. After the complete consumption of the starting material, the reaction mass was poured into ice-cold water and extracted using ethyl acetate. The organic layer was dried over anhydrous sodium sulfate and evaporated under vacuum. The crude product was purified using column chromatography with pet ether/ethyl acetate to obtain the pure compound. Yield: 5.4 g, 90%. Yellow crystalline solid, m.p. 55–56 °C. IR (neat, cm^{-1}): 2981, 1725, 1651, 1235, 1140, 1033. ^1H NMR (DMSO-d_6, 400 MHz, δ in ppm): 7.69–7.11 (m, 5H, Ar-H), 4.32 (q, 2H, $–CH_2$), 4.02 (s, 3H, N–CH_3), 1.33 (t, 3H, $–CH_3$, J = 7.2 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 161.8, 139.7, 128.1, 125.8, 125.3, 122.7, 121.0, 111.3, 110.0, 60.8, 32.0, 14.7. MS: m/z = 204.2 (M + H). Anal. Calcd. for $C_{12}H_{13}NO_2$: C, 70.92; H, 6.45; N, 6.89. Found: C, 70.90; H, 6.39; N, 6.85.

2.2.2. Procedure for synthesis of ethyl 5-fluoro-1-methyl-1H-indole-2-carboxylate (**5b**)

Intermediate **5b** was prepared by treating compound **4b** (6 g, 28.95 mmol) with a catalytic amount of *n*-TBAB, potassium carbonate (8 g, 57.91 mmol) and methyl iodide (5.6 mL, 86.87 mmol) in DMF (60 mL) by following the above mentioned procedure. Yield: 5.1 g, 85%. Orange crystalline solid, m.p. 73–74 °C. IR (neat, cm^{-1}): 2985, 1720, 1648, 1232, 1149, 1030. ^1H NMR (DMSO-d_6, 400 MHz, δ in ppm): 7.66–7.10 (m, 4H, Ar-H), 4.22 (q, 2H, $–CH_2$), 4.01 (s, 3H, N–CH_3), 1.30 (t, 3H, $–CH_3$, J = 7.2 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 161.7, 138.0, 126.1, 125.8, 125.3, 122.7, 122.0, 111.3, 111.1, 63.0, 32.0, 14.7. MS: m/z = 222.2 (M + H). Anal. Calcd. for $C_{12}H_{12}FNO_2$: C, 65.15; H, 5.47; N, 6.33. Found: C, 65.11; H, 5.44; N, 6.29.

2.2.3. Procedure for the synthesis of 1-methyl-1H-indole-2-carboxylic acid (**6a**)

The intermediate **5a** (5 g, 24.60 mmol) was taken in MeOH: THF (1:1) solvent mixture to which a solution of LiOH (3 g, 73.80 mmol) in distilled water was added drop wise at 0 °C. After the complete addition, the reaction mass was stirred at RT for about 3 h. TLC showed the completion of the reaction following which the solvent was removed in vacuum. Then, 10 mL distilled water was added to the residue, after which the unreacted ester, if any, was removed by ether extraction. The aqueous portion was then acidified using 50% HCl. The product precipitated on vigorous stirring was filtered off, washed with cold water and dried under vacuum. This filtered solid was pure and needed no further purification. Yield: 4.5 g, 90%. White solid, m.p. 208–209 °C. IR (neat, cm^{-1}): 2978, 1676, 1519, 1473, 1265, 1054, 1012, 730. ^1H NMR (DMSO-d_6, 400 MHz, δ in ppm): 10.6 (s, 1H, –OH), 7.68–7.10 (m, 5H, Ar-H), 3.98 (s, 3H, N–CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 163.5, 139.7, 129.0, 125.8, 125.1, 122.6, 120.8, 111.3, 109.9, 31.9. MS: m/z = 176.1 (M + H). Anal. Calcd. for $C_{10}H_9NO_2$: C, 68.56; H, 5.18; N, 8.00. Found: C, 68.51; H, 5.15; N, 7.98.

2.2.4. Procedure for the synthesis of 5-fluoro-1-methyl-1H-indole-2-carboxylic acid (**6b**)

Intermediate **6b** was prepared by treating compound **5b** (4.9 g, 22.14 mmol) with LiOH (2.8 g, 66.44 mmol) in MeOH: THF (1:1) mixture as per the above procedure. Yield: 4.3 g, 88% Off-white solid, m.p. 262–263 °C. IR (neat, cm^{-1}): 2981, 1676, 1520, 1473, 1271, 1238, 1054, 1013, 727. ^1H NMR (DMSO-d_6, 400 MHz, δ in ppm): 10.9 (s, 1H, –OH), 7.69–7.08 (m, 4H, Ar-H), 3.98 (s, 3H, N–CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 162.8, 158.8, 139.6, 135.5, 128.9, 114.4, 112.2, 112.2, 109.7, 31.9. MS: m/z = 194.2 (M + H). Anal. Calcd. for $C_{10}H_8FNO_2$: C, 62.18; H, 4.17; N, 7.25. Found: C, 62.14; H, 4.13; N, 7.21.

2.2.5. Procedure for the synthesis of N-cyclopropyl-1-methyl-1H-indole-2-carboxamide (**7a**)

To a stirred solution of **6a** (4.4 g, 25.11 mmol) in DMF (44 mL), cyclopropyl amine (2.9 g, 50.23 mmol) was added and the solution was cooled to 0 °C. Coupling agent HATU (10.5 g, 27.62 mmol) was added followed by the addition of base, di-isopropylethyl amine (13 mL, 75.35 mmol) and the reaction mixture was left for stirring for 16 h at RT. After the completion of the reaction as monitored by TLC, the reaction mixture was poured into ice-cold water and stirred well. The precipitated product was then filtered by suction, washed with cold water, dried and weighed. It was then recrystallized using ethanol. Yield: 4.1 g, 93%. White solid, m.p. 182–183 °C. IR (neat, cm^{-1}): 2977, 1628, 1534, 1458, 1278, 1053, 1009, 746. ^1H NMR (DMSO-d_6, 400 MHz, δ in ppm): 8.47 (s, 1H, –NH), 7.61–7.02 (m, 5H, Ar-H), 3.97 (s, 3H, N–CH_3), 2.85–2.83 (m, 1H, –CH), 0.72–0.56 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 163.5, 138.8, 132.6, 126.0, 123.9, 121.9, 120.5, 110.9, 104.8, 31.7, 23.2, 6.2. MS: m/z = 215.1 (M + H). Anal. Calcd. for $C_{13}H_{14}N_2O$: C, 72.87; H, 6.59; N, 13.07. Found: C, 72.85; H, 6.55; N, 13.03.

2.2.6. Procedure for the synthesis of N-cyclopropyl-5-fluoro-1-methyl-1H-indole-2-carboxamide (**7b**)

Intermediate **7b** was prepared according to the above mentioned procedure by treating **6b** (4.2 g, 21.74 mmol) with cyclopropyl amine (2.5 g, 43.48 mmol), HATU (9 g, 23.91 mmol) and DIPEA (11 mL, 65.22 mmol) in DMF (42 mL). Yield: 3.9 g, 93%. Off-white solid, m.p. 172–173 °C. IR (neat, cm^{-1}): 2979, 1628, 1537, 1460, 1278, 1054, 1013, 747, 646. ^1H NMR (DMSO-d_6, 400 MHz, δ in ppm): 8.49 (s, 1H, –NH), 7.54–6.98 (m, 4H, Ar-H), 3.95 (s, 3H, N-CH_3), 2.84–2.80 (m, 1H, –CH), 0.71–0.54 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 163.2, 159.0, 135.5, 134.1, 126.1, 112.3, 112.2, 112.2, 106.2, 32.0, 23.1, 6.2. MS: m/z = 233.2 (M + H). Anal. Calcd. for $C_{13}H_{13}FN_2O$: C, 67.23; H, 5.64; N, 12.06. Found: C, 67.19; H, 5.61; N, 12.01.

2.2.7. Procedure for the synthesis of N-cyclopropyl-3-formyl-1-methyl-1H-indole-2-carboxamide (**8a**)

To a dried two-neck round bottom flask containing DMF (3 mL, 37.33 mmol), phosphorous oxychloride ($POCl_3$) (4 mL, 37.33 mmol) was added dropwise through a syringe under argon at a temperature below 0 °C. After the formation of the iminium cation, a solution of intermediate **7a** (4 g, 18.66 mmol) in DMF (40 mL) was added and the entire

reaction mass was heated at 60 °C for 2 h. Reaction was monitored using TLC and after the complete consumption of the starting material, the reaction mixture was poured into crushed ice and stirred well. The formed solid was collected by filtration, washed with cold water, dried and used as such for the next step. Yield: 3.7 g, 93%. Red solid, m.p. 160–161 °C. IR (neat, cm^{-1}): 2970, 2864, 1639, 1527, 1390, 1285, 1054, 1012, 835, 748. ^1H NMR (DMSO-d_6, 400 MHz, δ in ppm): 9.92 (s, 1H, –CHO), 9.19 (s, 1H, –NH), 8.19–7.28 (m, 4H, Ar-H), 3.82 (s, 3H, N–CH_3), 2.97–2.92 (m, 1H, –CH), 0.78–0.60 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 184.0, 160.3, 143.0, 136.2, 124.0, 123.1, 122.7, 120.9, 113.9, 110.6, 30.8, 22.4, 5.3. MS: m/z = 243.3 (M + H). Anal. Calcd. for $C_{14}H_{14}N_2O_2$: C, 69.41; H, 5.82; N, 11.56. Found: C, 69.38; H, 5.79; N, 11.52.

2.2.8. Procedure for the synthesis of N-cyclopropyl-5-fluoro-3-formyl-1-methyl-1H-indole-2-carboxamide (**8b**)

The above procedure was followed for the synthesis of **8b** by treating **7b** (3.8 g, 16.36 mmol) with $POCl_3$ (3 mL, 32.72 mmol) and DMF (2.5 mL, 32.72 mmol). Yield: 3.5 g, 92%. Olive green solid, m.p. 192–193 °C. IR (neat, cm^{-1}): 2973, 2864, 1639, 1528, 1391, 1285, 1054, 1012, 836, 748, 645. ^1H NMR (DMSO-d_6, 400 MHz, δ in ppm): 9.89 (s, 1H, –CHO), 9.21 (s, 1H, –NH), 7.86–7.23 (m, 3H, Ar-H), 3.83 (s, 3H, N–CH_3), 2.96–2.92 (m, 1H, –CH), 0.78–0.60 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 185.1, 161.0, 158.6, 145.0, 134.0, 124.7, 124.6, 113.6, 113.6, 113.5, 32.2, 23.5, 6.3. MS: m/z = 261.1 (M + H). Anal. Calcd. for $C_{14}H_{13}FN_2O_2$: C, 64.61; H, 5.03; N, 10.76. Found: C, 64.58; H, 4.99; N, 10.71.

2.2.9. General Procedure for the synthesis of chalcone intermediates (**9a**–**v**)

To a stirred ethanolic solution of required aromatic acetophenone in a dry round bottom flask, 10% solution of NaOH in ethanol was added and stirred at RT for 30 min. The formylated intermediate **8a**/**8b** (0.2 g) was then added to the reaction mixture and stirring was continued for 2 h. Reaction completion was confirmed by running TLC following which the precipitated solid was cooled adequately. The solid formed was then filtered under vacuum, washed with cold ethanol, dried and recrystallized using ethanol. The obtained chalcone intermediates **9a**–**v** were suitably characterized and used as scaffolds for the final step.

2.3. Structural characterization data of intermediates **9a**–**v**

2.3.1. N-cyclopropyl-1-methyl-3-(3-oxo-3-phenylprop-1-enyl)-1H-indole-2-carboxamide (**9a**)

Yield: 0.16 g, 80%. Pale yellow solid, m.p. 181–182 °C. IR (neat, cm^{-1}): 3254, 1643, 1568, 1466, 1405, 1378, 1293, 1254, 1158, 1020, 844, 772, 743, 695, 646. ^1H NMR (DMSO-d_6, 400 MHz, δ in ppm): 9.03 (s, 1H, –NH), 8.22–8.08 (m, 3H, Ar-H), 8.000–7.961 (d, 1H, C═CH, J = 15.6 Hz), 7.67–7.56 (m, 5H, Ar-H), 7.433–7.394 (d, 1H, CH═C, J = 15.6 Hz), 7.36–7.32 (m, 1H, Ar-H), 3.80 (s, 3H, N–CH_3), 3.01–2.97 (m, 1H, –CH), 0.82–0.59 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 189.8, 162.4, 143.3, 141.8, 137.9, 135.7, 129.9, 129.4, 128.7, 126.2, 123.1, 121.6, 124.5, 122.8, 122.0, 118.9, 111.7, 31.7, 23.4, 6.3. MS: m/z = 345.2 (M + H). Anal. Calcd. for $C_{22}H_{20}N_2O_2$: C, 76.72; H, 5.85; N, 8.13. Found: C, 76.69; H, 5.82; N, 8.10.

2.3.2. N-cyclopropyl-3-(3-(4-fluorophenyl)-3-oxoprop-1-enyl)-1-methyl-1H-indole-2-carboxamide (**9b**)

Yield: 0.18 g, 90%. Pale yellow solid, m.p. 200–201 °C. IR (neat, cm^{-1}): 3252, 1640, 1574, 1539, 1466, 1407, 1379, 1287, 1222, 1157, 1017, 834, 743. ^1H NMR (DMSO-d_6, 400 MHz, δ in ppm): 9.04 (s, 1H, –NH), 8.22–8.09 (m, 3H, Ar-H), 8.028–7.989 (d, 1H, C═CH, J = 15.6 Hz), 7.63–7.51 (m, 5H, Ar–H), 7.430–7.391 (d, 1H, CH═C, J = 15.6 Hz), 3.78 (s, 3H, N–CH_3), 3.01–2.92 (m, 1H, –CH), 0.84–0.59 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 189.8, 169.6, 162.4, 143.5, 141.7, 135.7, 133.9, 133.8, 129.9, 128.6, 127.6, 122.8, 122.0, 120.2, 118.9, 117.4, 117.2, 111.7, 31.7, 23.4, 6.3. MS: m/z = 363.2 (M + H). Anal. Calcd. for $C_{22}H_{19}FN_2O_2$: C, 72.91; H, 5.28; N, 7.73. Found: C, 72.88; H, 5.26; N, 7.70.

2.3.3. 3-(3-(4-Chlorophenyl)-3-oxoprop-1-enyl)-N-cyclopropyl-1-methyl-1H-indole-2-carboxamide (**9c**)

Yield: 0.17 g, 85%. Yellow solid, m.p. 232–233 °C. IR (neat, cm^{-1}): 3249, 1652, 1572, 1539, 1462, 1428, 1390, 1374, 1279, 1220, 1034, 828, 785, 746, 694. ^1H NMR (DMSO-d_6, 400 MHz, δ in ppm): 9.02 (s, 1H, –NH), 8.19–8.07 (m, 3H, Ar-H), 7.972–7.933 (d, 1H, C═CH, J = 15.6 Hz), 7.67–7.44 (m, 5H, Ar-H), 7.410–7.371 (d, 1H, CH═C, J = 15.6 Hz), 3.78 (s, 3H, N–CH_3), 3.00–2.98 (m, 1H, –CH), 0.84–0.59 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 189.8, 162.4, 144.5, 143.7, 140.7, 135.8, 131.9, 131.4, 129.9, 128.6, 128.5, 128.4, 127.6, 122.8, 120.2, 119.6, 118.1, 111.7, 31.7, 23.4, 6.3. MS: m/z = 379.6 (M + H). Anal. Calcd. for $C_{22}H_{19}ClN_2O_2$: C, 69.75; H, 5.05; N, 7.39. Found: C, 69.72; H, 5.03; N, 7.36.

2.3.4. 3-(3-(4-Bromophenyl)-3-oxoprop-1-enyl)-N-cyclopropyl-1-methyl-1H-indole-2-carboxamide (**9d**)

Yield: 0.16 g, 80%. Pale brown solid, m.p. 207–208 °C. IR (neat, cm^{-1}): 3247, 1672, 1644, 1577, 1552, 1511, 1439, 1288, 1249, 1174, 1105, 934, 850, 811, 793, 728, 645. ^1H NMR (DMSO-d_6, 400 MHz, δ in ppm): 9.05 (s, 1H, –NH), 8.19–8.07 (m, 3H, Ar-H), 7.963–7.924 (d, 1H, C═CH, J = 15.6 Hz), 7.61–7.47 (m, 5H, Ar-H), 7.410–7.371 (d, 1H, CH═C, J = 15.6 Hz), 3.77 (s, 3H, N–CH_3), 3.00–2.97 (m, 1H, –CH), 0.84–0.59 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 189.8, 162.4, 144.5, 143.7, 135.8, 132.7, 132.1, 131.9, 131.7, 129.8, 129.0, 128.6, 127.6, 122.8, 120.2, 119.6, 118.1, 111.7, 31.7, 23.4, 6.3. MS: m/z = 424.1 (M + H). Anal. Calcd. for $C_{22}H_{19}BrN_2O_2$: C, 62.42; H, 4.52; N, 6.62. Found: C, 62.39; H, 4.50; N, 6.59.

2.3.5. N-cyclopropyl-3-(3-(4-methoxyphenyl)-3-oxoprop-1-enyl)-1-methyl-1H-indole-2-carboxamide (**9e**)

Yield: 0.18 g, 90%. White solid, m.p. 185–186 °C. IR (neat, cm^{-1}): 3399, 1655, 1638, 1579, 1554, 1511, 1438, 1294, 1253, 1222, 1111, 836, 818, 735, 701, 652. ^1H NMR (DMSO-d_6, 400 MHz, δ in ppm): 9.01 (s, 1H, –NH), 8.22–8.10 (m, 3H,

Ar-H), 7.97–7.931 (d, 1H, C═CH, J = 15.6 Hz), 7.683–7.644 (d, 1H, CH═C, J = 15.6 Hz), 7.62–7.02 (m, 5H, Ar-H), 3.86 (s, 3H, O–CH_3), 3.78 (s, 3H, N–CH_3), 3.00–2.98 (m, 1H, –CH), 0.80–0.61 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 189.8, 165.7, 162.5, 144.2, 143.6, 131.1, 130.5, 130.32, 128.8, 128.6, 127.5, 122.8, 120.4, 119.6, 118.1, 115.3, 114.8, 111.7, 54.4, 31.7, 23.4, 6.4. MS: m/z = 375.4 (M + H). Anal. Calcd. for $C_{23}H_{22}N_2O_3$: C, 73.78; H, 5.92; N, 7.48. Found: C, 7.75; H, 5.90; N, 7.45.

*2.3.6. N-cyclopropyl-1-methyl-3-(3-oxo-3-p-tolylprop-1-enyl)-1H-indole-2-carboxamide (**9f**)*

Yield: 0.18 g, 91%. Pale yellow solid, m.p. 174–175 °C. IR (neat, cm^{-1}): 3248, 1638, 1572, 1518, 1460, 1404, 1377, 1287, 1221, 1109, 1020, 823, 742. ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 9.02 (s, 1H, –NH), 8.21–8.00 (m, 3H, Ar-H), 7.985–7.946 (d, 1H, C═CH, J = 15.6 Hz), 7.66–7.37 (m, 5H, Ar-H), 7.363–7.324 (d, 1H, CH═C, J = 15.6 Hz), 3.79 (s, 3H, N–CH_3), 3.01–2.97 (m, 1H, –CH), 2.41 (s, 3H, –CH_3), 0.82–0.59 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 189.8, 162.5, 144.3, 144.2, 143.6, 133.9, 129.8, 129.3, 129.1, 128.8, 128.6, 127.5, 122.8, 120.4, 119.6, 118.1, 111.7, 31.7, 23.4, 21.5, 6.4. MS: m/z = 359.2 (M + H). Anal. Calcd. for $C_{23}H_{22}N_2O_2$: C, 77.07; H, 6.19; N, 7.82. Found: C, 77.04; H, 6.16; N, 7.79.

*2.3.7. N-cyclopropyl-3-(3-(3-fluorophenyl)-3-oxoprop-1-enyl)-1-methyl-1H-indole-2-carboxamide (**9g**)*

Yield: 0.17 g, 85%. Yellow solid, m.p. 211–212 °C. IR (neat, cm^{-1}): 3253, 1643, 1575, 1543, 1466, 1409, 1379, 1288, 1230, 1157, 1018, 834, 745. ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 9.04 (s, 1H, –NH), 8.14–7.97 (m, 3H, Ar–H), 7.969–7.930 (d, 1H, C═CH, J = 15.6 Hz), 7.87–7.72 (m, 5H, Ar–H), 7.660–7.699 (d, 1H, CH═C, J = 15.6 Hz), 3.78 (s, 3H, N–CH_3), 3.01–2.94 (m, 1H, –CH), 0.83–0.59 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 189.8, 163.5, 162.4, 144.6, 143.6, 139.3, 131.3, 129.9, 128.6, 127.6, 125.2, 122.8, 122.0, 120.2, 119.1, 118.6, 115.4, 111.7, 31.7, 23.4, 6.3. MS: m/z = 363.2 (M + H). Anal. Calcd. for $C_{22}H_{19}FN_2O_2$: C, 72.91; H, 5.28; N, 7.73. Found: C, 72.88; H, 5.26; N, 7.70.

*2.3.8. 3-(3-(3-Chlorophenyl)-3-oxoprop-1-enyl)-N-cyclopropyl-1-methyl-1H-indole-2-carboxamide (**9h**)*

Yield: 0.17 g, 86%. White solid, m.p. 203–204 °C. IR (neat, cm^{-1}): 3248, 1659, 1560, 1536, 1461, 1431, 1392, 1374, 1278, 1220, 1035, 829, 785, 744, 696. ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 9.04 (s, 1H, –NH), 8.24–8.09 (m, 3H, Ar–H), 7.984–7.945 (d, 1H, C═CH, J = 15.6 Hz), 7.80–7.53 (m, 5H, Ar–H), 7.463–7.424 (d, 1H, CH═C, J = 15.6 Hz), 3.78 (s, 3H, N–CH_3), 3.00–2.98 (m, 1H, –CH), 0.83–0.57 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 189.8, 162.4, 144.5, 143.7, 139.1, 136.0, 134.5, 130.9, 130.1, 129.9, 128.7, 128.4, 127.6, 122.8, 120.2, 119.6, 118.1, 111.7, 31.7, 23.4, 6.3. MS: m/z = 379.6 (M + H). Anal. Calcd. for $C_{22}H_{19}ClN_2O_2$: C, 69.75; H, 5.05; N, 7.39. Found: C, 69.72; H, 5.03; N, 7.36.

*2.3.9. 3-(3-(3-Bromophenyl)-3-oxoprop-1-enyl)-N-cyclopropyl-1-methyl-1H-indole-2-carboxamide (**9i**)*

Yield: 0.16 g, 81%. Pale yellow solid, m.p. 196–197 °C. IR (neat, cm^{-1}): 3249, 1670, 1651, 1577, 1552, 1513, 1439, 1274, 1249, 1174, 1105, 938, 850, 811, 793, 728, 647. ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 9.03 (s, 1H, –NH), 8.19–8.07 (m, 3H, Ar-H), 7.971–7.932 (d, 1H, C═CH, J = 15.6 Hz), 7.56–7.42 (m, 5H, Ar-H), 7.396–7.357 (d, 1H, CH═C, J = 15.6 Hz), 3.77 (s, 3H, N–CH_3), 3.00–2.97 (m, 1H, –CH), 0.84–0.59 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 189.8, 162.4, 144.5, 143.7, 141.3, 137.1, 133.4, 131.9, 129.8, 129.0, 128.6, 127.6, 125.0, 122.8, 120.2, 119.7, 118.1, 111.7, 31.7, 23.4, 6.3. MS: m/z = 424.4 (M + H). Anal. Calcd. for $C_{22}H_{19}BrN_2O_2$: C, 62.42; H, 4.52; N, 6.62. Found: C, 62.39; H, 4.50; N, 6.59.

*2.3.10. N-cyclopropyl-3-(3-(3-methoxyphenyl)-3-oxoprop-1-enyl)-1-methyl-1H-indole-2-carboxamide (**9j**)*

Yield: 0.16 g, 80%. Pale yellow solid, m.p. 190–191 °C. IR (neat, cm^{-1}): 3397, 1655, 1640, 1581, 1555, 1511, 1439, 1286, 1250, 1222, 1111, 836, 818, 735, 705, 653. ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 9.01 (s, 1H, –NH), 7.97–7.931 (d, 1H, C═CH, J = 15.6 Hz), 7.682–7.643 (d, 1H, CH═C, J = 15.6 Hz), 3.87 (s, 3H, O–CH_3), 3.78 (s, 3H, N–CH_3), 3.02–2.97 (m, 1H, –CH), 0.80–0.61 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 189.8, 163.7, 162.5, 144.2, 143.6, 138.2, 130.5, 129.7, 128.8, 127.5, 125.3, 121.3, 122.8, 120.2, 119.7, 118.1, 115.3, 111.7, 54.4, 31.7, 23.4, 6.4. MS: m/z = 375.4 (M + H). Anal. Calcd. for $C_{23}H_{22}N_2O_3$: C, 73.78; H, 5.92; N, 7.48. Found: C, 73.75; H, 5.90; N, 7.45.

*2.3.11. N-cyclopropyl-1-methyl-3-(3-oxo-3-m-tolylprop-1-enyl)-1H-indole-2-carboxamide (**9k**)*

Yield: 0.17 g, 85%. Yellow solid, m.p. 189–190 °C. IR (neat, cm^{-1}): 3248, 1638, 1573, 1520, 1461, 1405, 1377, 1287, 1221, 1109, 1021, 825, 745. ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 9.03 (s, 1H, –NH), 8.36–8.17 (m, 3H, Ar-H), 7.974–7.935 (d, 1H, C═CH, J = 15.6 Hz), 7.77–7.54 (m, 5H, Ar-H), 7.447–7.408 (d, 1H, CH═C, J = 15.6 Hz), 3.79 (s, 3H, N–CH_3), 3.01–2.97 (m, 1H, –CH), 2.43 (s, 3H, –CH_3), 0.81–0.59 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 189.8, 162.5, 140.7, 138.8, 138.6, 137.9, 137.6, 133.7, 129.1, 129.0, 125.9, 124.6, 124.5, 122.8, 122.0, 118.0, 111.7, 110.9, 31.7, 23.4, 21.5, 6.4. MS: m/z = 359.2 (M + H). Anal. Calcd. for $C_{23}H_{22}N_2O_2$: C, 77.07; H, 6.19; N, 7.82. Found: C, 77.04; H, 6.16; N, 7.80.

*2.3.12. N-cyclopropyl-5-fluoro-1-methyl-3-(3-oxo-3-phenylprop-1-enyl)-1H-indole-2-carboxamide (**9l**)*

Yield: 0.19 g, 94%. White solid, m.p. 196–197 °C. IR (neat, cm^{-1}): 3251, 1641, 1571, 1540, 1484, 1459, 1383, 1279, 1173, 1130, 846, 797, 771, 695, 646. ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 9.04 (s, 1H, –NH), 8.12–8.00 (m, 3H, Ar-H), 7.941–7.902 (d, 1H, C═CH, J = 15.6 Hz), 7.68–7.62 (m, 3H, Ar-H), 7.604–7.565 (d, 1H, CH═C, J = 15.6 Hz), 7.55–7.22 (m, 2H, Ar-H), 3.78 (s, 3H, N–CH_3), 2.98–2.95 (m, 1H, –CH), 0.80–0.57 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 189.8, 162.4, 158.7, 144.3, 140.5,

139.1, 135.6, 130.4, 129.9, 129.4, 128.7, 128.6, 128.1, 127.5, 118.7, 113.3, 112.8, 112.7, 31.7, 23.4, 6.3. MS: m/z = 363.4 (M + H). Anal. Calcd. for $C_{22}H_{19}FN_2O_2$: C, 72.91; H, 5.28; N, 7.73. Found: C, 72.89; H, 5.25; N, 7.71.

*2.3.13. N-cyclopropyl-5-fluoro-3-(3-(4-fluorophenyl)-3-oxoprop-1-enyl)-1-methyl-1H-indole-2-carboxamide (**9m**)*

Yield: 0.18 g, 91%. Orange solid, m.p. 207–208 °C. IR (neat, cm^{-1}): 3254, 1642, 1575, 1541, 1509, 1485, 1406, 1383, 1277, 1159, 1131, 1019, 835, 794, 643. ^1H NMR (DMSO-d_6, 400 MHz, δ in ppm): 9.04 (s, 1H, –NH), 8.25–8.03 (m, 3H, Ar-H), 7.947–7.908 (d, 1H, C═CH, J = 15.6 Hz), 7.68–7.65 (m, 1H, Ar-H), 7.607–7.568 (d, 1H, CH═C, J = 15.6 Hz), 7.38–7.22 (m, 3H, Ar-H), 3.78 (s, 3H, N–CH_3), 2.99–2.94 (m, 1H, –CH), 0.84–0.57 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 189.7, 169.6, 162.4, 158.8, 144.6, 140.3, 135.6, 133.7, 133.2, 130.7, 128.6, 127.6, 118.9, 117.2, 117.2, 113.3, 112.8, 112.7, 31.7, 23.4, 6.3. MS: m/z = 381.2 (M + H). Anal. Calcd. for $C_{22}H_{18}F_2N_2O_2$: C, 69.46; H, 4.77; N, 7.36. Found: C, 69.44; H, 4.75; N, 7.34.

*2.3.14. 3-(3-(4-Chlorophenyl)-3-oxoprop-1-enyl)-N-cyclopropyl-5-fluoro-1-methyl-1H-indole-2-carboxamide (**9n**)*

Yield: 0.17 g, 85%. Yellow solid, m.p. 235–236 °C. IR (neat, cm^{-1}): 3252, 1571, 1540, 1483, 1409, 1284, 1242, 1222, 1171, 1132, 1024, 847, 791, 667. ^1H NMR (DMSO-d_6, 400 MHz, δ in ppm): 9.03 (s, 1H, –NH), 8.32–8.11 (m, 3H, Ar-H), 7.927–7.888 (d, 1H, C═CH, J = 15.6 Hz), 7.73–7.65 (m, 1H, Ar-H), 7.558–7.519 (d, 1H, CH═C, J = 15.6 Hz), 7.40–7.19 (m, 3H, Ar-H), 3.78 (s, 3H, N–CH_3), 3.00–2.95 (m, 1H, –CH), 0.83–0.56 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 189.8, 162.4, 158.8, 144.5, 143.7, 140.7, 135.8, 132.3, 131.9, 131.4, 128.6, 128.5, 128.4, 127.6, 119.6, 113.3, 112.8, 112.3, 31.7, 23.4, 6.3. MS: m/z = 397.4 (M + H). Anal. Calcd. for $C_{22}H_{18}ClFN_2O_2$: C, 66.58; H, 4.57; N, 7.06. Found: C, 66.55; H, 4.54; N, 7.03.

*2.3.15. 3-(3-(4-Bromophenyl)-3-oxoprop-1-enyl)-N-cyclopropyl-5-fluoro-1-methyl-1H-indole-2-carboxamide (**9o**)*

Yield: 0.16 g, 81%. Pale yellow solid, m.p. 240–241 °C. IR (neat, cm^{-1}): 3253, 1655, 1635, 1589, 1531, 1477, 1369, 1294, 1276, 1247, 1175, 1106, 930, 838, 815, 797, 727, 667. ^1H NMR (DMSO-d_6, 400 MHz, δ in ppm): 9.03 (s, 1H, –NH), 8.32–8.11 (m, 3H, Ar-H), 7.869–7.830 (d, 1H, C═CH, J = 15.6 Hz), 7.70–7.61 (m, 1H, Ar-H), 7.571–7.532 (d, 1H, CH═C, J = 15.6 Hz), 7.31–7.13 (m, 3H, Ar-H), 3.78 (s, 3H, N–CH_3), 3.00–2.94 (m, 1H, –CH), 0.83–0.57 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 189.8, 162.4, 158.8, 144.5, 140.6, 135.8, 132.7, 132.1, 131.9, 131.7, 130.3, 129.0, 128.6, 127.6, 119.6, 113.3, 112.8, 112.3, 31.7, 23.4, 6.3. MS: m/z = 442.3 (M + H). Anal. Calcd. for $C_{22}H_{18}BrFN_2O_2$: C, 59.88; H, 4.11; N, 6.35. Found: C, 59.85; H, 4.08; N, 6.32.

*2.3.16. N-cyclopropyl-5-fluoro-3-(3-(4-methoxyphenyl)-3-oxoprop-1-enyl)-1-methyl-1H-indole-2-carboxamide (**9p**)*

Yield: 0.19 g, 93%. Pale yellow solid, m.p. 221–222 °C. IR (neat, cm^{-1}): 3243, 1658, 1640, 1582, 1557, 1479, 1371, 1298, 1277, 1257, 1225, 1175, 1111, 932, 837, 817, 798, 695. ^1H NMR (DMSO-d_6, 400 MHz, δ in ppm): 9.03 (s, 1H, –NH), 8.17–7.94 (m, 3H, Ar-H), 7.932–7.893 (d, 1H, C═CH, J = 15.6 Hz), 7.69–7.66 (m, 1H, Ar-H), 7.628–7.589 (d, 1H, CH═C, J = 15.6 Hz), 7.29–7.07 (m, 3H, Ar-H), 3.87 (s, 3H, O–CH_3), 3.79 (s, 3H, N–CH_3), 3.01–2.97 (m, 1H, –CH), 0.82–0.60 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 189.7, 165.7, 162.5, 157.8, 144.2, 140.6, 131.8, 131.1, 130.5, 130.3, 128.8, 127.5, 118.4, 115.3, 114.8, 113.3, 112.8, 112.3, 54.4, 31.7, 23.4, 6.4. MS: m/z = 393.4 (M + H). Anal. Calcd. for $C_{23}H_{21}FN_2O_3$: C, 70.40; H, 5.39; N, 7.14. Found: C, 70.38; H, 5.35; N, 7.11.

*2.3.17. N-cyclopropyl-5-fluoro-1-methyl-3-(3-oxo-3-p-tolylprop-1-enyl)-1H-indole-2-carboxamide (**9q**)*

Yield: 0.18 g, 90%. Yellow solid, m.p. 199–200 °C. IR (neat, cm^{-1}): 3247, 1639, 1571, 1541, 1487, 1407, 1386, 1282, 1246, 1220, 1166, 1130, 789, 646. ^1H NMR (DMSO-d_6, 400 MHz, δ in ppm): 9.03 (s, 1H, –NH), 8.04–7.99 (m, 3H, Ar-H), 7.928–7.889 (d, 1H, C═CH, J = 15.6 Hz), 7.68–7.64 (m, 1H, Ar-H), 7.593–7.554 (d, 1H, CH═C, J = 15.6 Hz), 7.36–7.22 (m, 3H, Ar-H), 3.77 (s, 3H, N–CH_3), 2.99–2.95 (m, 1H, –CH), 2.39 (s, 3H, –CH_3), 0.80–0.57 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 189.7, 162.5, 157.8, 144.3, 144.2, 140.6, 133.9, 130.7, 129.3, 129.1, 128.8, 128.6, 127.5, 118.4, 113.3, 112.8, 112.3, 31.7, 23.4, 21.5, 6.4. MS: m/z = 377.6 (M + H). Anal. Calcd. for $C_{23}H_{21}FN_2O_2$: C, 73.39; H, 5.62; N, 7.44. Found: C, 73.36; H, 5.60; N, 7.41.

*2.3.18. N-cyclopropyl-5-fluoro-3-(3-(3-fluorophenyl)-3-oxoprop-1-enyl)-1-methyl-1H-indole-2-carboxamide (**9r**)*

Yield: 0.17 g, 86%. White solid, m.p. 210–211 °C. IR (neat, cm^{-1}): 3255, 1642, 1575, 1541, 1509, 1486, 1406, 1381, 1277, 1160, 1131, 1019, 836, 794, 645. ^1H NMR (DMSO-d_6, 400 MHz, δ in ppm): 9.04 (s, 1H, –NH), 8.30–8.10 (m, 3H, Ar-H), 7.988–7.949 (d, 1H, C═CH, J = 15.6 Hz), 7.82–7.89 (m, 1H, Ar-H), 7.682–7.643 (d, 1H, CH═C, J = 15.6 Hz), 7.50–7.31 (m, 3H, Ar-H), 3.78 (s, 3H, N–CH_3), 2.99–2.94 (m, 1H, –CH), 0.84–0.57 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 189.8, 163.5, 162.4, 158.8, 144.6, 140.3, 139.3, 131.3, 130.2, 128.6, 127.6, 125.2, 122.0, 118.7, 115.4, 113.3, 112.8, 112.7, 31.7, 23.4, 6.3. MS: m/z = 381.2 (M + H). Anal. Calcd. for $C_{22}H_{18}F_2N_2O_2$: C, 69.46; H, 4.77; N, 7.36. Found: C, 69.44; H, 4.75; N, 7.34.

*2.3.19. 3-(3-(3-Chlorophenyl)-3-oxoprop-1-enyl)-N-cyclopropyl-5-fluoro-1-methyl-1H-indole-2-carboxamide (**9s**)*

Yield: 0.18 g, 91%. Pale yellow solid, m.p. 233–234 °C. IR (neat, cm^{-1}): 3253, 1574, 1540, 1484, 1410, 1284, 1244, 1223, 1171, 1132, 1024, 847, 794, 667. ^1H NMR (DMSO-d_6, 400 MHz, δ in ppm): 9.03 (s, 1H, –NH), 8.49–8.31 (m, 3H, Ar-H), 7.940–7.901 (d, 1H, C═CH, J = 15.6 Hz), 7.87–7.78 (m, 1H, Ar-H), 7.610–7.571 (d, 1H, CH═C, J = 15.6 Hz), 7.53–7.28 (m, 3H, Ar-H), 3.78 (s, 3H, N–CH_3), 3.00–2.95 (m, 1H, –CH), 0.83–0.56 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 189.8, 162.4, 157.8, 144.5, 143.6, 139.1, 136.0, 134.5, 132.3, 130.9, 130.1, 128.7, 128.4, 127.6, 119.6, 113.3, 112.8, 112.3, 31.7, 23.4, 6.3. MS: m/z = 397.4 (M + H). Anal. Calcd. for $C_{22}H_{18}ClFN_2O_2$: C, 66.58; H, 4.57; N, 7.06. Found: C, 66.55; H, 4.54; N, 7.03.

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*2.3.20. 3-(3-(3-Bromophenyl)-3-oxoprop-1-enyl)-N-cyclopropyl-5-fluoro-1-methyl-1H-indole-2-carboxamide (**9t**)*

Yield: 0.17 g, 85%. Pale brown solid, m.p. 235–236 °C. IR (neat, cm^{-1}): 3261, 1653, 1649, 1589, 1530, 1477, 1369, 1294, 1266, 1247, 1179, 1102, 934, 837, 815, 797, 725, 668. ^1H NMR (DMSO-d_6, 400 MHz, δ in ppm): 9.03 (s, 1H, –NH), 8.41–8.15 (m, 3H, Ar-H), 7.822–7.783 (d, 1H, C═CH, J = 15.6 Hz), 7.76–7.69 (m, 1H, Ar-H), 7.555–7.516 (d, 1H, CH═C, J = 15.6 Hz), 7.43–7.06 (m, 3H, Ar-H), 3.78 (s, 3H, N–CH_3), 3.00–2.94 (m, –1H, –CH), 0.84–0.57 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 189.8, 162.4, 157.8, 144.5, 141.3, 140.1, 137.2, 133.4, 131.9, 130.3, 129.0, 128.6, 127.6, 124.6, 119.6, 113.3, 112.8, 112.3, 31.7, 23.4, 6.3. MS: m/z = 442.3 (M + H). Anal. Calcd. for $C_{22}H_{18}BrFN_2O_2$: C, 59.88; H, 4.11; N, 6.35. Found: C, 59.85; H, 4.08; N, 6.32.

*2.3.21. N-cyclopropyl-5-fluoro-3-(3-(3-methoxyphenyl)-3-oxoprop-1-enyl)-1-methyl-1H-indole-2-carboxamide (**9u**)*

Yield: 0.16 g, 80%. Pale yellow solid, m.p. 215–216 °C. IR (neat, cm^{-1}): 3246, 1660, 1633, 1582, 1558, 1482, 1371, 1298, 1275, 1257, 1224, 1175, 1112, 932, 837, 817, 798, 693. ^1H NMR (DMSO-d_6, 400 MHz, δ in ppm): 9.03 (s, 1H, –NH), 8.17–7.94 (m, 3H, Ar-H), 7.87–7.831 (d, 1H, C═CH, J = 15.6 Hz), 7.70–7.65 (m, 1H, Ar-H), 7.619–7.580 (d, 1H, CH═C, J = 15.6 Hz), 7.42–7.11(m, 3H, Ar-H), 3.87 (s, 3H, O–CH_3), 3.79 (s, 3H, N–CH_3), 3.01–2.96 (m, 1H, –CH), 0.82–0.59 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 189.8, 163.7, 162.5, 157.8, 144.2, 140.6, 138.2, 131.1, 130.5, 128.8, 127.5, 125.3, 121.3, 118.4, 115.3, 113.3, 112.8, 112.3, 54.4, 31.7, 23.4, 6.4. MS: m/z = 393.4 (M + H). Anal. Calcd. for $C_{23}H_{21}FN_2O_3$: C, 70.40; H, 5.39; N, 7.14. Found: C, 70.38; H, 5.35; N, 7.11.

*2.3.22. N-cyclopropyl-5-fluoro-1-methyl-3-(3-oxo-3-m-tolylprop-1-enyl)-1H-indole-2-carboxamide (**9v**)*

Yield: 0.17 g, 85%. Off-white solid, m.p. 220–221 °C. IR (neat, cm^{-1}): 3247, 1640, 1571, 1541, 1487, 1407, 1387, 1282, 1246, 1222, 1164, 1130, 787, 646. ^1H NMR (DMSO-d_6, 400 MHz, δ in ppm): 9.03 (s, 1H, –NH), 8.12–7.97 (m, 3H, Ar-H), 7.933–7.894 (d, 1H, C═CH, J = 15.6 Hz), 7.72–7.66 (m, 1H, Ar-H), 7.585–7.546 (d, 1H, CH═C, J = 15.6 Hz), 7.36–7.21 (m, 3H, Ar-H), 3.77 (s, 3H, N–CH_3), 2.99–2.95 (m, 1H, –CH), 2.38 (s, 3H, –CH_3), 0.80–0.58 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 189.8, 162.5, 158.1, 140.7, 138.8, 138.6, 137.9, 137.6, 133.7, 129.1, 129.0, 127.9, 125.1, 119.0, 113.5, 112.5, 112.3, 31.7, 23.4, 21.5, 6.4. MS: m/z = 377.6 (M + H). Anal. Calcd. for $C_{23}H_{21}FN_2O_2$: C, 73.39; H, 5.62; N, 7.44. Found: C, 73.36; H, 5.60; N, 7.41.

*2.3.23. General procedure for the synthesis of target compounds (**10a**–**v**)*

A mixture comprising of the scaffold **9a**–**v** (0.15 g) and guanidine hydrochloride (2 equivalents) in ethanol was taken in a dry round bottom flask to which ethanolic NaOH was added with stirring at 0 °C. The reaction mass was refluxed for 12 h at 80 °C. Completion of the reaction was determined using TLC and after complete consumption of the starting material, the reaction mass was kept for cooling. It was then poured into crushed ice with stirring and filtered in the cold condition. The obtained solid was washed with cold water, dried properly and recrystallized using ethanol to afford the pure target compounds **10a**–**v**.

2.4. The structural characterization of the final compounds is listed below

*2.4.1. 3-(2-Amino-6-phenylpyrimidin-4-yl)-N-cyclopropyl-1-methyl-1H-indole-2-carboxamide (**10a**)*

IR (neat, cm^{-1}): 3203, 2978, 1636, 1568, 1464, 1405, 1378, 1285, 1220, 1158, 1020, 844, 772, 743, 695, 645. ^1H NMR (400 MHz, DMSO-d_6, δ in ppm): 9.16 (s, 1H, –NH), 8.47–7.21 (m, 10H, Ar-H), 6.67 (s, 2H, –NH_2), 3.81 (s, 3H, N–CH_3), 3.01–2.99 (m, 1H, –CH), 0.73–0.48 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 164.3, 164.0, 163.9, 162.4, 143.7, 139.5, 136.3, 133.6, 130.7, 129.2, 128.4, 126.9, 126.0, 123.1, 121.6, 118.4, 115.2, 110.9, 102.5, 31.6, 23.4, 6.0. MS: m/z = 384.2 (M + H). Anal. Calcd. for $C_{23}H_{21}N_5O$: C, 72.04; H, 5.52; N, 18.26. Found: C, 71.99; H, 5.48; N, 18.24.

*2.4.2. 3-(2-Amino-6-(4-fluorophenyl)pyrimidin-4-yl)-N-cyclopropyl-1-methyl-1H-indole-2-carboxamide (**10b**)*

IR (neat, cm^{-1}): 3207, 2984, 1640, 1575, 1541, 1509, 1466, 1407, 1379, 1287, 1221, 1157, 1016, 834, 743. ^1H NMR (400 MHz, DMSO-d_6, δ in ppm): 9.15 (s, 1H, –NH), 8.48–7.05 (m, 9H, Ar-H), 6.69 (s, 2H, –NH_2), 3.81 (s, 3H, N–CH_3), 3.00–2.96 (m, 1H, –CH), 0.70–0.46 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 164.3, 163.8, 163.9, 162.4, 162.0, 143.7, 139.5, 133.6, 130.4, 129.8, 128.4, 123.1, 121.6, 118.4, 117.6, 117.3, 115.2, 110.9, 102.5, 31.6, 23.4, 6.0. MS: m/z = 402.2 (M + H). Anal. Calcd. for $C_{23}H_{20}FN_5O$: C, 68.81; H, 5.02; N, 17.45. Found: C, 68.77; H, 4.91; N, 17.39.

*2.4.3. 3-(2-Amino-6-(4-chlorophenyl)pyrimidin-4-yl)-N-cyclopropyl-1-methyl-1H-indole-2-carboxamide (**10c**)*

IR (neat, cm^{-1}): 3346, 3217, 1658, 1560, 1536, 1465, 1431, 1390, 1376, 1278, 1220, 1035, 829, 784, 744, 721, 696. ^1H NMR (400 MHz, DMSO-d_6, δ in ppm): 9.13 (s, 1H, –NH), 8.45–7.18 (m, 9H, Ar-H), 6.72 (s, 2H, –NH_2), 3.80 (s, 3H, N–CH_3), 3.00–2.98 (m, 1H, –CH), 0.76–0.48 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 164.3, 163.9, 164.0, 162.3, 143.9, 139.5, 135.4, 133.5, 132.5, 130.7, 129.2, 128.4, 127.1, 123.1, 121.6, 118.5, 115.3, 110.3, 102.5, 31.4, 23.4, 6.0. MS: m/z = 418.4 (M + H). Anal. Calcd. for $C_{23}H_{20}ClN_5O$: C, 66.10; H, 4.82; N, 16.76. Found: C, 66.04; H, 4.77; N, 16.70.

*2.4.4. 3-(2-Amino-6-(4-bromophenyl)pyrimidin-4-yl)-N-cyclopropyl-1-methyl-1H-indole-2-carboxamide (**10d**)*

IR (neat, cm^{-1}): 3328, 3202, 1578, 1564, 1538, 1486, 1465, 1393, 1285, 1222, 1160, 1135, 1106, 1009, 825, 795, 742, 663. ^1H NMR (400 MHz, DMSO-d_6, δ in ppm): 9.11 (s, 1H, –NH), 8.45–7.18 (m, 9H, Ar-H), 6.71 (s, 2H, –NH_2), 3.80 (s, 3H, N–CH_3), 3.02–2.98 (m, 1H, –CH), 0.72–0.46 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 164.3, 163.9, 163.9, 162.3, 143.7, 140.5, 135.5, 133.6, 132.3, 131.9, 129.6, 129.1, 124.7, 123.1, 121.6, 118.5, 115.3, 110.3, 102.5, 31.4, 23.4, 6.0. MS: m/z = 463.4 (M + H). Anal. Calcd. for

$C_{23}H_{20}BrN_5O$: C, 59.75; H, 4.36; N, 15.15. Found: C, 59.71; H, 4.29; N, 15.10.

*2.4.5. 3-(2-Amino-6-(4-methoxyphenyl)pyrimidin-4-yl)-N-cyclopropyl-1-methyl-1H-indole-2-carboxamide (**10e**)*

IR (neat, cm^{-1}): 3331, 3209, 3009, 1638, 1605, 1569, 1538, 1512, 1287, 1253, 1222, 1176, 1134, 1111, 1028, 832, 796, 743. ^1H NMR (400 MHz, DMSO-d_6, δ in ppm): 9.18 (s, 1H, –NH), 8.42–7.07 (m, 9H, Ar-H), 6.59 (s, 2H, $–NH_2$), 3.83 (s, 3H, $N–CH_3$), 3.81 (s, 3H, $O–CH_3$), 3.01–2.94 (m, 1H, –CH), 0.75–0.48 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 164.3, 163.9, 163.7, 162.3, 160.2, 143.5, 140.6, 135.6, 127.8, 127.1, 125.4, 123.1, 121.6, 118.6, 115.3, 113.7, 113.2, 109.8, 102.7, 53.8, 31.4, 23.4, 6.0. MS: m/z = 414.2 (M + H). Anal. Calcd. for $C_{24}H_{23}N_5O_2$: C, 69.72; H, 5.61; N, 16.94. Found: C, 69.66; H, 5.58; N, 16.89.

*2.4.6. 3-(2-Amino-6-p-tolylpyrimidin-4-yl)-N-cyclopropyl-1-methyl-1H-indole-2-carboxamide (**10f**)*

IR (neat, cm^{-1}): 3346, 3031, 1638, 1570, 1513, 1464, 1404, 1377, 1286, 1220, 1110, 1020, 820, 742. ^1H NMR (400 MHz, DMSO-d_6, δ in ppm): 9.17 (s, 1H, –NH), 8.43–7.10 (m, 9H, Ar-H), 6.69 (s, 2H, $–NH_2$), 3.83 (s, 3H, $N–CH_3$), 3.00–2.97 (m, 1H, –CH), 2.38 (s, 3H, $–CH_3$), 0.72–0.50 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 164.2, 163.9, 163.7, 162.2, 140.5, 139.7, 138.1, 135.2, 133.5, 129.8, 126.8, 125.94, 125.8, 123.1, 121.6, 118.4, 115.2, 110.9, 104.3, 31.3, 23.4, 21.4, 6.0. MS: m/z = 398.2 (M + H). Anal. Calcd. for $C_{24}H_{23}N_5O$: C, 72.52; H, 5.83; N, 17.62. Found: C, 72.48; H, 5.79; N, 17.60.

*2.4.7. 3-(2-Amino-6-(3-fluorophenyl)pyrimidin-4-yl)-N-cyclopropyl-1-methyl-1H-indole-2-carboxamide (**10g**)*

IR (neat, cm^{-1}): 3341, 3294, 1613, 1572, 1466, 1440, 1403, 1379, 1285, 1220, 1160, 1108, 1020, 877, 818, 789, 743, 702. ^1H NMR (400 MHz, DMSO-d_6, δ in ppm): 9.15 (s, 1H, –NH), 8.43–7.09 (m, 9H, Ar-H), 6.74 (s, 2H, $–NH_2$), 3.81 (s, 3H, $N–CH_3$), 3.00–2.96 (m, 1H, –CH), 0.70–0.46 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 164.3, 164.0, 163.9, 162.4, 162.0, 141.7, 139.5, 138.4, 133.8, 130.5, 122.8, 123.4, 123.1, 121.6, 118.4, 116.6, 116.2, 115.2, 110.9, 102.6, 31.6, 23.4, 6.0. MS: m/z = 402.2 (M + H). Anal. Calcd. for $C_{23}H_{20}FN_5O$: C, 68.81; H, 5.02; N, 17.45. Found: C, 68.77; H, 4.92; N, 17.38.

*2.4.8. 3-(2-Amino-6-(3-chlorophenyl)pyrimidin-4-yl)-N-cyclopropyl-1-methyl-1H-indole-2-carboxamide (**10h**)*

IR (neat, cm^{-1}): 3357, 3288, 1658, 1611, 1568, 1530, 1465, 1431, 1394, 1376, 1278, 1219, 1035, 829, 785, 744, 721, 698. ^1H NMR (400 MHz, DMSO-d_6, δ in ppm): 9.16 (s, 1H, –NH), 8.48–7.18 (m, 9H, Ar-H), 6.78 (s, 2H, $–NH_2$), 3.80 (s, 3H, $N–CH_3$), 3.01–2.99 (m, 1H, –CH), 0.73–0.46 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 164.3, 163.6, 163.5, 161.8, 141.6, 140.2, 138.5, 135.6, 134.7, 131.1, 129.3, 127.3, 124.0, 123.1, 121.7, 118.3, 116.6, 111.1, 102.9, 31.6, 23.4, 6.0. MS: m/z = 418.4 (M + H). Anal. Calcd. for $C_{23}H_{20}ClN_5O$: C, 66.10; H, 4.82; N, 16.76. Found: C, 66.04; H, 4.77; N, 16.71.

*2.4.9. 3-(2-Amino-6-(3-bromophenyl)pyrimidin-4-yl)-N-cyclopropyl-1-methyl-1H-indole-2-carboxamide (**10i**)*

IR (neat, cm^{-1}): 3343, 3206, 1579, 1564, 1530, 1465, 1393, 1285, 1222, 1160, 1070, 1009, 825, 795, 743, 663. ^1H NMR (400 MHz, DMSO-d_6, δ in ppm): 9.14 (s, 1H, –NH), 8.45–7.11 (m, 9H, Ar-H), 6.77 (s, 2H, $–NH_2$), 3.81 (s, 3H, $N–CH_3$), 3.00–2.98 (m, 1H, –CH), 0.74–0.46 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 164.3, 163.9, 163.9, 162.4, 143.7, 140.5, 135.5, 135.0, 132.8, 131.4, 129.9, 127.5, 124.7, 123.1, 121.6, 118.4, 115.3, 109.3, 103.2, 31.4, 23.4, 6.0. MS: m/z = 463.2 (M + H). Anal. Calcd. for $C_{23}H_{20}BrN_5O$: C, 59.75; H, 4.36; N, 15.15. Found: C, 59.71; H, 4.29; N, 15.09.

*2.4.10. 3-(2-Amino-6-(3-methoxyphenyl)pyrimidin-4-yl)-N-cyclopropyl-1-methyl-1H-indole-2-carboxamide (**10j**)*

IR (neat, cm^{-1}): 3325, 3005, 1611, 1569, 1465, 1285, 1223, 1160, 1044, 967, 869, 842, 787, 743, 701. ^1H NMR (400 MHz, DMSO-d_6, δ in ppm): 9.15 (s, 1H, –NH), 8.45–7.07 (m, 9H, Ar-H), 6.68 (s, 2H, $–NH_2$), 3.83 (s, 3H, $N–CH_3$), 3.80 (s, 3H, $O–CH_3$), 3.02–2.97 (m, 1H, –CH), 0.72–0.47 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 164.3, 163.9, 163.7, 162.3, 160.2, 143.5, 140.6, 135.6, 133.2, 130.4, 123.1, 121.6, 120.2, 118.6, 115.3, 114.9, 110.5, 109.8, 102.7, 53.9, 31.4, 23.5, 6.0. MS: m/z = 414.2 (M + H). Anal. Calcd. for $C_{24}H_{23}N_5O_2$: C, 69.72; H, 5.61; N, 16.94. Found: C, 69.66; H, 5.57; N, 16.89.

*2.4.11. 3-(2-Amino-6-m-tolylpyrimidin-4-yl)-N-cyclopropyl-1-methyl-1H-indole-2-carboxamide (**10k**)*

IR (neat, cm^{-1}): 3205, 3047, 1637, 1570, 1465, 1403, 1378, 1286, 1219, 1161, 1021, 967, 871, 790, 742, 703. ^1H NMR (400 MHz, DMSO-d_6, δ in ppm): 9.18 (s, 1H, –NH), 8.47–7.18 (m, 9H, Ar-H), 6.66 (s, 2H, $–NH_2$), 3.81 (s, 3H, $N–CH_3$), 3.00–2.98 (m, 1H, –CH), 2.40 (s, 3H, $–CH_3$), 0.72–0.50 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 164.2, 164.1, 164.0, 162.9, 138.3, 138.1, 137.0, 136.3, 131.4, 129.1, 127.5, 125.5, 124.2, 123.8, 123.1, 121.6, 112.2, 110.9, 104.1, 31.4, 23.4, 21.6, 6.0. MS: m/z = 398.2 (M + 1). Anal. Calcd. for $C_{24}H_{23}N_5O$: C, 72.52; H, 5.83; N, 17.62. Found: C, 72.48; H, 5.79; N, 17.60.

*2.4.12. 3-(2-Amino-6-phenylpyrimidin-4-yl)-N-cyclopropyl-5-fluoro-1-methyl-1H-indole-2-carboxamide (**10l**)*

IR (neat, cm^{-1}): 3210, 3187, 1630, 1570, 1540, 1484, 1455, 1382, 1278, 1173, 1130, 846, 796, 771, 695, 645. ^1H NMR (400 MHz, DMSO-d_6, δ in ppm): 9.15 (s, 1H, –NH), 8.33–7.16 (m, 9H, Ar-H), 6.72 (s, 2H, $–NH_2$), 3.79 (s, 3H, $N–CH_3$), 3.00–2.99 (m, 1H, –CH), 0.73–0.45 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 164.3, 164.0, 163.9, 162.4, 159.8, 138.1, 133.6, 130.7, 129.2, 126.9, 126.0, 125.9, 112.3, 112.2, 112.0, 111.7, 111.7, 108.3, 108.1, 103.2, 31.6, 23.5, 5.9. MS: m/z = 402.0 (M + H). Anal. Calcd. for $C_{23}H_{20}FN_5O$: C, 68.81; H, 5.02; N, 17.45. Found: C, 68.77; H, 4.90; N, 17.39.

*2.4.13. 3-(2-Amino-6-(4-fluorophenyl)pyrimidin-4-yl)-N-cyclopropyl-5-fluoro-1-methyl-1H-indole-2-carboxamide (**10m**)*

IR (neat, cm^{-1}): 3326, 3291, 1602, 1574, 1541, 1509, 1484, 1406, 1382, 1277, 1157, 1130, 1019, 835, 793. ^1H NMR

(400 MHz, DMSO-d_6, δ in ppm): 9.15 (s, 1H, –NH), 8.32–7.13 (m, 8H, Ar-H), 6.73 (s, 2H, $–NH_2$), 3.79 (s, 3H, N–CH_3), 3.01–2.98 (m, 1H, –CH), 0.73–0.44 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 164.3, 164.0, 163.5, 162.4, 162.0, 159.9, 141.7, 139.5, 136.4, 130.7, 129.2, 128.4, 118.4, 117.6, 117.3, 113.6, 112.4, 110.1, 110.0, 102.5, 31.6, 23.4, 6.0. MS: m/z = 420.2 (M + H). Anal. Calcd. for $C_{23}H_{19}F_2N_5O$: C, 65.86; H, 4.57; N, 16.70. Found: C, 65.80; H, 4.52; N, 16.64.

*2.4.14. 3-(2-Amino-6-(4-chlorophenyl)pyrimidin-4-yl)-N-cyclopropyl-5-fluoro-1-methyl-1H-indole-2-carboxamide (**10n**)*

IR (neat, cm^{-1}): 3218, 3117, 1571, 1540, 1483, 1408, 1280, 1241, 1222, 1171, 1132, 1023, 845, 791, 667. ^{1}H NMR (400 MHz, DMSO-d_6, δ in ppm): 9.14 (s, 1H, –NH), 8.32–7.14 (m, 8H, Ar–H), 6.76 (s, 2H, $–NH_2$), 3.79 (s, 3H, N–CH_3), 3.00–2.97 (m, 1H, –CH), 0.74–0.44 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 164.3, 163.9, 163.5, 162.3, 159.7, 141.5, 140.2, 135.4, 133.5, 132.5, 130.7, 129.2, 128.4, 127.1, 118.2, 113.6, 112.4, 110.1, 102.5, 31.4, 23.4, 6.0. MS: m/z = 436.0 (M + H). Anal. Calcd. for $C_{23}H_{19}ClFN_5O$: C, 63.38; H, 4.39; N, 16.07. Found: C, 63.33; H, 4.37; N, 16.03.

*2.4.15. 3-(2-Amino-6-(4-bromophenyl)pyrimidin-4-yl)-N-cyclopropyl-5-fluoro-1-methyl-1H-indole-2-carboxamide (**10o**)*

IR (neat, cm^{-1}): 3334, 3286, 1629, 1577, 1535, 1483, 1399, 1283, 1242, 1130, 1071, 1008, 829, 795. ^{1}H NMR (400 MHz, DMSO-d_6, δ in ppm): 9.18 (s, 1H, –NH), 8.32–7.11 (m, 8H, Ar-H), 6.79 (s, 2H, $–NH_2$), 3.81 (s, 3H, N–CH_3), 3.01–2.99 (m, 1H, –CH), 0.74–0.46 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 164.3, 163.9, 163.9, 162.3, 159.8, 140.7, 139.1, 135.7, 133.6, 132.3, 131.9, 129.6, 129.1, 124.7, 118.2, 113.6, 112.4, 110.1, 102.5, 31.4, 23.4, 6.0. MS: m/z = 481.2 (M + H). Anal. Calcd. for $C_{23}H_{19}BrFN_5O$: C, 57.51; H, 3.99; N, 14.58. Found: C, 57.48; H, 3.91; N, 14.53.

*2.4.16. 3-(2-Amino-6-(4-methoxyphenyl)pyrimidin-4-yl)-N-cyclopropyl-5-fluoro-1-methyl-1H-indole-2-carboxamide (**10p**)*

IR (neat, cm^{-1}): 3211, 3195, 1638, 1570, 1541, 1484, 1408, 1284, 1251, 1222, 1181, 1130, 1046, 845, 789, 742. ^{1}H NMR (400 MHz, DMSO-d_6, δ in ppm): 9.19 (s, 1H, –NH), 9.18–7.08 (m, 8H, Ar-H), 6.67 (s, 2H, $–NH_2$), 3.83 (s, 3H, O–CH_3), 3.79 (s, 3H, N–CH_3), 3.00–2.97 (m, 1H, –CH), 0.73–0.48 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 164.3, 163.9, 163.7, 162.3, 160.2, 159.8, 140.5, 138.8, 135.6, 127.8, 127.1, 125.4, 118.2, 113.7, 113.6, 113.2, 112.4, 111.4, 102.7, 53.8, 31.4, 23.4, 6.0. MS: m/z = 432.2 (M + H). Anal. Calcd. for $C_{24}H_{22}FN_5O_2$: C, 66.81; H, 5.14; N, 16.23. Found: C, 66.78; H, 5.09; N, 16.19.

*2.4.17. 3-(2-Amino-6-p-tolylpyrimidin-4-yl)-N-cyclopropyl-5-fluoro-1-methyl-1H-indole-2-carboxamide (**10q**)*

IR (neat, cm^{-1}): 3327, 3214, 1636, 1571, 1542, 1487, 1406, 1386, 1282, 1246, 1220, 1166, 1131, 789. ^{1}H NMR (400 MHz, DMSO-d_6, δ in ppm): 9.16 (s, 1H, –NH), 8.32–7.13 (m, 8H, Ar-H), 6.67 (s, 2H, $–NH_2$), 3.79 (s, 3H, N–CH_3), 3.01–2.99 (m, 1H, –CH), 2.38 (s, 3H, –CH_3), 0.74–0.46 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 164.2, 163.9, 163.7, 162.2, 159.7, 157.4, 140.5, 138.1, 135.2, 133.5, 129.8, 126.8, 125.9, 125.8, 112.2, 112.2, 111.9, 111.7, 108.3, 102.8, 31.5, 23.4, 21.4, 5.9. MS: m/z = 416.1 (M + H). Anal. Calcd. for $C_{24}H_{22}FN_5O$: C, 69.38; H, 5.34; N, 16.86. Found; C, 69.33; H, 5.30; N, 16.82.

*2.4.18. 3-(2-Amino-6-(3-fluorophenyl)pyrimidin-4-yl)-N-cyclopropyl-5-fluoro-1-methyl-1H-indole-2-carboxamide (**10r**)*

IR (neat, cm^{-1}): 3215, 3119, 1624, 1572, 1485, 1406, 1335, 1281, 1248, 1220, 1192, 1130, 1023, 932, 882, 845, 749, 669. ^{1}H NMR (400 MHz, DMSO-d_6, δ in ppm): 9.13 (s, 1H, –NH), 8.30–7.13 (m, 8H, Ar-H), 6.77 (s, 2H, $–NH_2$), 3.77 (s, 3H, N–CH_3), 3.02-2.98 (m, 1H, –CH), 0.70–0.46 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 164.3, 164.0, 163.5, 162.4, 162.0, 159.9, 141.7, 139.5, 136.4, 133.8, 130.5, 122.8, 118.40 116.6, 116.2, 113.6, 110.1, 109.9, 103.1, 31.6, 23.4, 6.0. MS: m/z = 420.2 (M + H). Anal. Calcd. for $C_{23}H_{19}F_2N_5O$: C, 65.86; H, 4.57; N, 16.70. Found: C, 65.80; H, 4.52; N, 16.64.

*2.4.19. 3-(2-Amino-6-(3-chlorophenyl)pyrimidin-4-yl)-N-cyclopropyl-5-fluoro-1-methyl-1H-indole-2-carboxamide (**10s**)*

IR (neat, cm^{-1}): 3209, 3125, 1567, 1539, 1483, 1405, 1280, 1242, 1222, 1174, 1130, 1023, 845, 789, 667. ^{1}H NMR (400 MHz, DMSO-d_6, δ in ppm): 9.17 (s, 1H, –NH), 8.34–7.15 (m, 8H, Ar–H), 6.79 (s, 2H, $–NH_2$), 3.79 (s, 3H, N–CH_3), 3.03–3.00 (m, 1H, –CH), 0.75–0.46 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 164.3, 163.6, 163.5, 161.9, 159.8, 141.6, 140.2, 136.0, 135.6, 134.7, 131.1, 129.3, 127.3, 124.0, 116.3, 113.7, 113.1, 110.0, 102.9, 31.5, 23.5, 6.0. MS: m/z = 436.2 (M + H). Anal. Calcd. for $C_{23}H_{19}ClFN_5O$: C, 63.38; H, 4.39; N, 16.07. Found: C, 63.33; H, 4.37; N, 16.03.

*2.4.20. 3-(2-Amino-6-(3-bromophenyl)pyrimidin-4-yl)-N-cyclopropyl-5-fluoro-1-methyl-1H-indole-2-carboxamide (**10t**)*

IR (neat, cm^{-1}): 3213, 3186, 1623, 1574, 1538, 1482, 1404, 1279, 1242, 1222, 1172, 1130, 1069, 844, 787, 667. ^{1}H NMR (400 MHz, DMSO-d_6, δ in ppm): 9.18 (s, 1H, –NH), 8.30–7.11 (m, 8H, Ar-H), 6.78 (s, 2H, $–NH_2$), 3.79 (s, 3H, N–CH_3), 3.03–2.99 (m, 1H, –CH), 0.75–0.44 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 164.3, 163.9, 163.9, 162.4, 159.8, 141.8, 140.5, 135.5, 135.0, 132.8, 131.4, 129.9, 127.5, 124.7, 117.5, 113.4, 112.5, 109.6, 103.2, 31.4, 23.4, 6.0. MS: m/z = 481.2 (M + H). Anal. Calcd. for $C_{23}H_{19}BrFN_5O$: C, 57.51; H, 3.99; N, 14.58. Found: C, 57.47; H, 3.91; N, 14.53.

*2.4.21. 3-(2-Amino-6-(3-methoxyphenyl)pyrimidin-4-yl)-N-cyclopropyl-5-fluoro-1-methyl-1H-indole-2-carboxamide (**10u**)*

IR (neat, cm^{-1}): 3208, 3191, 1633, 1570, 1541, 1484, 1406, 1284, 1251, 1224, 1176, 1130, 1044, 845, 789, 746. ^{1}H NMR (400 MHz, DMSO-d_6, δ in ppm): 9.16 (s, 1H, –NH), 8.62–7.04 (m, 8H, Ar-H), 6.72 (s, 2H, $–NH_2$), 3.84 (s, 3H, N–CH_3), 3.78 (s, 3H, O–CH_3), 3.02–2.98 (m, 1H, –CH), 0.73–0.46 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 164.3, 163.9, 163.7, 162.3, 160.2, 159.9, 141.4, 139.9, 135.8, 133.3, 130.4, 120.2, 117.4, 114.9, 113.6, 112.5, 110.7, 109.1, 102.7, 53.9, 31.4, 23.5, 6.0. MS: m/z = 432.2 (M + H). Anal. Calcd. for $C_{24}H_{22}FN_5O_2$: C, 66.81; H, 5.14; N, 16.23. Found: C, 66.78; H, 5.09; N, 16.19.

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*2.4.22. 3-(2-Amino-6-m-tolylpyrimidin-4-yl)-N-cyclopropyl-5-fluoro-1-methyl-1H-indole-2-carboxamide (**10v**)*

IR (neat, cm^{-1}): 3322, 3214, 1632, 1571, 1542, 1484, 1406, 1380, 1282, 1246, 1220, 1166, 1129, 789. ^1H NMR (400 MHz, DMSO-d_6, δ in ppm): 9.18 (s, 1H, –NH), 8.34–7.14 (m, 8H, Ar-H), 6.70 (s, 2H, $–NH_2$), 3.79 (s, 3H, $N–CH_3$), 3.01–2.99 (m, 1H, –CH), 2.40 (s, 3H, $–CH_3$), 0.75–0.50 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 164.2, 164.1, 164.0, 162.9, 159.8, 138.6, 138.1, 137.0, 134.2, 131.4, 129.1, 127.5, 125.5, 124.2, 117.4, 113.7, 112.2, 111.6, 104.1, 31.4, 23.4, 21.6, 6.0. MS: m/z = 416.2 (M + H). Anal. Calcd. for $C_{24}H_{22}FN_5O$: C, 69.38; H, 5.34; N, 16.86. Found; C, 69.33; H, 5.30; N, 16.82.

3. Pharmacology

3.1. Cell lines, chemicals and culture medium

HeLa (Human cervix carcinoma), HepG2 (Liver, Rhesus monkey) and MCF-7 (Human Breast carcinoma) cell lines were procured from the National Centre for Cell Sciences (NCCS), Pune, India. 3-(4,5-dimethyl thiazol-2-yl)-5-diphenyl tetrazolium bromide (MTT), Foetal Bovine Serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM) and Trypsin were obtained from Sigma Aldrich Co., St. Louis, USA, EDTA, glucose and antibiotics from Hi-Media Laboratories Ltd., Mumbai and dimethyl sulfoxide (DMSO) and propanol from Merck Ltd., Mumbai, India. Stock cells were cultured in DMEM supplemented with 10% inactivated Foetal Bovine Serum (FBS), penicillin (100 IU/mL), streptomycin (100 μg/mL) and amphotericin B (5 μg/mL) in a humidified atmosphere of 5% CO_2 at 37 °C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^3 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

3.2. In vitro cytotoxic activity

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0×10^5 cells/mL using DMEM containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 mL of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 μL of different test concentrations of test drugs were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37 °C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the drug solutions in the wells were discarded and 50 μL of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37 °C in 5% CO_2 atmosphere. The supernatant was removed and 100 μL of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula,

$$\% \text{ Growth inhibition} = 100 - \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100$$

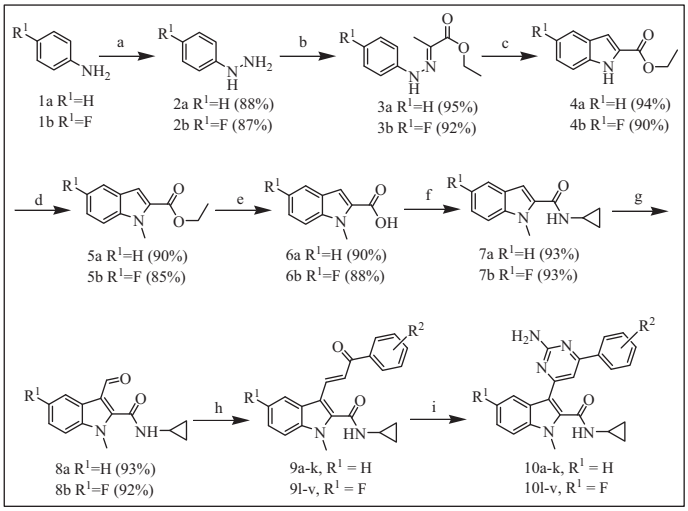
3.3. Anti-microbial studies

The disc diffusion method for antimicrobial susceptibility testing was carried out according to the standard method [33]. Nutrient agar (20 mL) was poured into each sterile Petri dish after injecting cultures (100 μL) of microorganisms and medium was distributed in the Petri dish homogeneously. Compounds were filtered with a pore size of 0.45 μm for sterilization. All the compounds were dissolved in DMSO of 5 mg/mL. Empty sterilized discs of 6 mm (Schleicher and Schuell, No. 2668, Germany) were impregnated with 10 μg of compounds. The discs were placed on agar plates, and the plates were incubated at 37 °C for 24 h. The culture suspensions were prepared and adjusted by comparing against 0.3 McFarland turbidity standard tubes. The inhibition zones formed on the medium were evaluated in millimetre (mm). The negative solvent control (DMSO) did not show any antimicrobial activity. The studies were performed in triplicate and the average reading was taken. The inhibition zones were compared with those of reference disc, Ciprofloxacin for antibacterial and Fluconazole for antifungal activity.

4. Results and discussion

4.1. Chemistry

The synthetic route of the intermediate and target compounds is outlined in Scheme 1. The core indole-2-ester (**4a/4b**) moiety was conveniently prepared using the Fischer-indole protocol [34]. The indole-NH was then methylated using potassium carbonate (K_2CO_3) as base and *tetra-n*-butyl ammonium bromide (*n*-TBAB) as phase transfer catalyst to give intermediates **5a** and **5b**. The intermediates **6a**/**6b** were synthesized by the hydrolysis of the ester functional group in **5a**/**5b**, using lithium hydroxide (LiOH) as base in methanolic THF medium. The obtained indole-2-acid was then coupled with cyclopropyl amine using HATU (1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluoro phosphate) as the coupling agent and di-isopropylethyl amine (DIPEA) as the base to give indole-2-amides **7a**/**7b** in excellent yields. In the next step, an aldehyde functionality was introduced at position-3 of the indole ring by following the Vilsmeier–Haack formylation process [35] to yield intermediates **8a**/**8b**. The chalcones **9a**–**v** were synthesized by subjecting the formylated intermediates **8a**/**8b** to conventional base-catalysed Claisen–Schmidt condensation reaction with acetophenone derivatives. Different acetophenones with various substitutions at position-3 and position-4 of the phenyl ring were employed to check their effect on biological activity. The substitutions included electron withdrawing groups like halogens and electron donating groups like methoxy and methyl groups. These chalcones were then cyclized to form indole-pyrimidine hybrids **10a**–**v** using guanidine hydrochloride in alcoholic NaOH medium under refluxing



Scheme 1 Synthetic route for indole based pyrimidine hybrids, Reagents and conditions: (a) HCl, $NaNO_2$, $SnCl_2$, −5 °C to RT O/N. (b) Ethyl pyruvate, cat. HOAc, EtOH, 80 °C 1 h. (c) PPA, Toluene, 100 °C, 5 h. (d) K_2CO_3, n-TBAB, DMF, MeI, RT, 12 h. (e) LiOH, MeOH:THF, RT, 3 h. (f) HATU, DIPEA, cyclopropylamine, DMF, RT, 16 h. (g) DMF, $POCl_3$, −5 to 60 °C, 2 h, H_2O. (h) NaOH, substituted acetophenones, EtOH, RT, 2 h. (i) Guanidine hydrochloride, NaOH, EtOH, 80 °C, 12 h.

condition. A library of twenty-two new compounds were synthesized by following the above mentioned procedure. All the reactions were monitored using thin layer chromatography and all the newly synthesized compounds were purified either by recrystallization or by column chromatographic techniques.

The structures of all the synthesized intermediates and final compounds were established by various spectral techniques *viz*. ^{1}H NMR, ^{13}C NMR, mass spectrometry and elemental data. Intermediates **6a**/**6b** were confirmed by the formation of broad peaks in the range of δ 12.4–12.1 ppm in their ^{1}H NMR spectra which corresponds to the –OH proton. In the ^{13}C NMR spectrum, the ─C═O carbon of –COOH group in **6a** and **6b** resonates at δ 163.5 and 162.8 ppm, respectively. The amide –NH in intermediates **7a**/**7b** appears as a singlet at around δ 8.47 ppm in the ^{1}H NMR spectrum. Further, the four aliphatic protons of the cyclopropyl ring appear as a multiplet whereas the junction –CH of the cyclopropyl ring gives a multiplet due to coupling with the neighbour protons. In the ^{13}C NMR spectra of **7a** and **7b**, the methylene carbon of the cyclopropyl ring appears at around δ 6.2 ppm whereas the junction carbon resonates at around δ 23.1 ppm. The formylation at position-3 of the indole ring was clearly established by the disappearance of an aromatic proton and the appearance of a new sharp singlet at around δ 9.92 ppm (due to –CHO group) in the ^{1}H NMR spectra of **8a** and **8b**. The presence of –CHO group was further confirmed by the appearance of a peak at around δ 184.0 ppm in their ^{13}C NMR spectra. Subsequent conversion of these formylated intermediates into different chalcones **9a**–**v** was also confirmed by NMR spectral studies. The olefinic protons in these chalcones resonate as two distinct doublets with the coupling constant (*J*) value in the range of 15.4–15.8 Hz. Also, these alkenyl protons appear in the downfield region (δ 7.9–7.5 ppm) of the spectrum. Both of these evidences strongly indicate that the hydrogens of the olefin have attained trans (*E*) configuration. In addition, the absence of a proton peak corresponding to the aldehydic group further supports the formation of respective chalcones. The cyclization of these pre-final intermediates (**9a**–**v**) to their corresponding target compounds **10a**–**v** was confirmed in the ^{1}H NMR spectrum by the formation of a new singlet peak in the range of δ 6.64–6.80 ppm owing to the presence of an amino group. Moreover, the complete disappearance of peaks due to olefinic protons also supports the proposed final structure of these molecules. The chemical structure of all the final molecules was further established by their ^{13}C NMR spectra. Additionally, the mass spectra are also in analogy with the structural molecular mass giving M + H peak *viz*. in the positive mode. The complete analytical and spectral data of all the intermediates and title compounds are discussed in the experimental section and their physical data are presented in Table 1.

4.2. Anti-proliferative studies

The synthesized compounds were evaluated for their ability to inhibit the growth of HepG2 (hepatocellular liver carcinoma), MCF-7 (human breast adenocarcinoma), HeLa (human cervical adenocarcinoma) and the non-tumourigenic Vero (African green monkey kidney epithelial cells) cell lines using the MTT assay [36]. The *in vitro* antiproliferative activity results are summarized in Table 2. MCF-7 and HepG2 cell lines were found to be sensitive as most of the synthesized compounds displayed higher growth inhibition on these two cells. Those compounds which inhibit the cancer cell growth to 70% or more could be considered as highly potent agents. Nature of substituents and the structural features aided us in deducing the structure-activity relationship (SAR). In accordance, it was found that compounds **10f**, **10o** and **10v** were the most active as they portrayed highest cytotoxic activity against all the three cell lines. They also depicted superior activity as compared to standard Doxorubicin and were twice as potent when compared to 5-Fluorouracil. Compounds **10f** and **10v** have an electron releasing methyl substitutent at the para (4-Me) and meta position (3-Me) of the phenyl ring (R^2) attached to

Table 1 Physical data of the target compounds (**10a–v**).

Code	R^1	R^2	Molecular formula	Molecular weight	Nature of compound	Melting point (°C)	Yield (%)
10a	H	H	$C_{23}H_{21}N_5O$	383.45	Off-white solid	118–119	91
10b	H	4-F	$C_{23}H_{20}FN_5O$	401.44	Off-white solid	105–106	75
10c	H	4-Cl	$C_{23}H_{20}ClN_5O$	417.89	Pale yellow solid	125–126	82
10d	H	4-Br	$C_{23}H_{20}BrN_5O$	462.34	Yellow solid	121–122	73
10e	H	4-OMe	$C_{24}H_{23}N_5O_2$	413.47	Pale yellow solid	95–96	90
10f	H	4-Me	$C_{24}H_{23}N_5O$	397.47	Yellow solid	126–127	87
10g	H	3-F	$C_{23}H_{20}FN_5O$	401.44	Yellow solid	108–109	91
10h	H	3-Cl	$C_{23}H_{20}ClN_5O$	417.89	Off-white solid	163–164	81
10i	H	3-Br	$C_{23}H_{20}BrN_5O$	462.34	Pale yellow solid	110–111	68
10j	H	3-OMe	$C_{24}H_{23}N_5O_2$	413.47	Pale yellow solid	115–116	76
10k	H	3-Me	$C_{24}H_{23}N_5O$	397.47	Off-white solid	125–126	80
10l	F	H	$C_{23}H_{20}FN_5O$	401.44	Off-white solid	129–130	87
10m	F	4-F	$C_{23}H_{19}F_2N_5O$	419.43	Off-white solid	115–116	83
10n	F	4-Cl	$C_{23}H_{19}ClFN_5O$	435.88	Brown solid	187–188	75
10o	F	4-Br	$C_{23}H_{19}BrFN_5O$	480.33	Yellow solid	189–190	76
10p	F	4-OMe	$C_{24}H_{22}FN_5O_2$	431.46	Pale yellow solid	118–119	82
10q	F	4-Me	$C_{24}H_{22}FN_5O$	415.46	Off-white solid	124–125	90
10r	F	3-F	$C_{23}H_{19}F_2N_5O$	419.43	Off-white solid	111–112	80
10s	F	3-Cl	$C_{23}H_{19}ClFN_5O$	435.88	Off-white solid	115–116	84
10t	F	3-Br	$C_{23}H_{19}BrFN_5O$	480.33	Yellow solid	140–141	61
10u	F	3-OMe	$C_{24}H_{22}FN_5O_2$	431.46	Pale yellow solid	117–118	59
10v	F	3-Me	$C_{24}H_{22}FN_5O$	415.46	Off-white solid	106–107	81

Table 2 Cytotoxicity of compounds **10a–v** against three human cancer cell lines (at 10 μM) and against noncancerous Vero cell line (at 50 μM).

Compound code	% Growth-inhibitory activity [a]			
	HeLa	HepG2	MCF-7	Vero
10a	32.59 ± 0.3	64.47 ± 1.7	68.29 ± 1.4	–
10b	21.28 ± 2.4	**88.33 ± 1.6**	**82.00 ± 0.6**	–
10c	62.25 ± 3.7	**90.62 ± 0.3**	**81.69 ± 0.6**	10.46 ± 2.6
10d	40.85 ± 6.8	**70.29 ± 1.1**	59.80 ± 1.7	–
10e	27.98 ± 1.0	48.87 ± 0.6	31.77 ± 0.6	–
10f	**70.60 ± 1.9**	**93.80 ± 0.1**	**86.97 ± 0.4**	12.76 ± 3.4
10g	62.78 ± 2.5	**77.29 ± 1.4**	**71.53 ± 3.6**	15.03 ± 2.1
10h	28.68 ± 2.6	**81.88 ± 0.6**	**72.39 ± 0.9**	–
10i	30.49 ± 0.3	50.35 ± 0.6	37.35 ± 2.3	–
10j	21.76 ± 0.2	40.72 ± 1.3	34.91 ± 1.9	–
10k	62.04 ± 1.6	**82.80 ± 2.0**	**82.98 ± 0.1**	–
10l	13.35 ± 2.7	56.34 ± 1.1	46.63 ± 1.9	–
10m	14.35 ± 4.0	42.65 ± 0.6	33.74 ± 4.2	–
10n	9.63 ± 2.3	7.94 ± 2.5	10.06 ± 0.5	–
10o	**82.51 ± 0.5**	**91.97 ± 1.9**	**86.91 ± 0.4**	17.28 ± 0.3
10p	15.30 ± 6.5	49.94 ± 1.1	20.73 ± 0.5	–
10q	14.35 ± 0.8	**84.16 ± 3.2**	**79.17 ± 2.7**	–
10r	21.58 ± 2.8	**77.41 ± 0.6**	**75.09 ± 1.5**	–
10s	35.68 ± 4.4	**76.59 ± 2.2**	**71.49 ± 1.8**	–
10t	46.44 ± 1.9	**81.38 ± 1.6**	56.40 ± 1.4	–
10u	60.99 ± 1.2	**93.95 ± 0.2**	**90.02 ± 0.1**	8.87 ± 1.4
10v	**80.59 ± 0.9**	**93.31 ± 0.3**	**89.65 ± 0.1**	22.97 ± 3.4
Doxorubicin	77.1 ± 2.4	65.3 ± 0.8	88.1 ± 0.3	46.1 ± 0.1
5-Fluorouracil	45.0 ± 3.3	42.0 ± 0.8	35.0 ± 2.2	22.1 ± 3.0

– Not determined.
The % growth inhibition values > 70 are highlighted in bold.
[a] All values are expressed as mean ± SEM (n = 3). Experiment was performed in triplicate.

pyrimidine unit respectively whereas compound **10o** comprises of bromo substituent at R^2. However, compounds **10k** (3-Me) and **10q** (4-Me) at R^2 have displayed selective cytotoxic inhibition on HepG2 and MCF-7 cells. Also, methoxy groups (at R^2) did not impart much activity except for compound **10u** (3-OMe). In terms of compounds with electron attracting

Table 3 Anti-microbial activity of the compounds (**10a–v**) at a concentration of 10 μg/disc.

Compound codes	Zone of inhibition in mm					
	Antibacterial activity				Antifungal activity	
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
10a	09	10	08	10	10	10
10b	**24**	**26**	**27**	**25**	**24**	**25**
10c	**26**	**26**	**28**	**26**	**27**	**27**
10d	18	19	17	16	19	18
10e	08	09	10	11	10	09
10f	08	08	09	10	09	08
10g	**29**	**28**	**25**	**27**	**25**	**26**
10h	**24**	**25**	**25**	**24**	**24**	**25**
10i	20	21	20	18	20	20
10j	08	08	09	08	10	10
10k	12	14	13	15	14	13
10l	16	14	18	14	15	15
10m	**28**	**26**	**25**	**25**	**26**	**25**
10n	**27**	**28**	**28**	**26**	**27**	**27**
10o	19	20	20	22	23	21
10p	14	16	16	18	16	19
10q	20	21	20	22	21	23
10r	**28**	**26**	**27**	**26**	**24**	**25**
10s	**24**	**26**	**22**	**25**	**26**	**24**
10t	18	17	20	23	20	19
10u	16	15	18	16	17	15
10v	20	21	21	20	18	17
Ciprofloxacin	27	26	28	25	–	–
Flucanazole	–	–	–	–	27	26

The % growth inhibition values > 70 are highlighted in bold.

groups, compounds **10b**, **10g** and **10r** with fluoro substitution (R^2) and compounds **10c**, **10h** and **10s** with chloro substitution (R^2) showed selective inhibition on HepG2 and MCF-7 cells whereas **10d** and **10t** having bromo group (R^2) exhibited cytotoxicity only on HepG2 cells. Unsusbstituted hybrids (R^2), **10a** and **10l** have not shown much activity, signifying the need of a substitution on the phenyl ring for enhanced activity. These data also suggest that in the case of compounds **10a–10k** (R^1 = H), R^2 substitution at position-4 and in compounds **10l–10v** (R^1 = F), R^2 substitution at position-3 of the phenyl ring have aided in improving the cytotoxic activity. It is also worthy to mention that all the active compounds displayed negligible growth inhibition on Vero cells indicating their non-toxic nature. The synthesized molecules can be further investigated to develop potential leads since majority of the active molecules are more potent as compared to the positive control drugs, Doxorubicin and 5-Fluorouracil.

4.3. Anti-microbial studies

The antimicrobial activity was determined using the disc diffusion method [33] by measuring the zone of inhibition in millimetre (mm). All the compounds (**10a–u**) were screened *in-vitro* at a concentration of 10 μg/disc for their antibacterial activity against two Gram-positive strains (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram-negative strains (*Escherichia coli* and *Pseudomonas aeruginosa*). The antifungal evaluation was carried out against *Candida albicans* and *Aspergillus niger* at a concentration of 10 μg/disc. Standard antibacterial drug ciprofloxacin (10 μg/disc) and antifungal drug fluconazole (10 μg/disc) were also tested under similar conditions against these organisms. All the synthesized compounds exhibited significant antibacterial and antifungal activities. Each experiment was performed in triplicate and the average reading was taken (Table 3). The activity was classified as highly active (≥26 mm), moderately active (11–25 mm) and least active (< 11 mm). In view of the values obtained, compounds **10b**, **10c**, **10g**, **10h**, **10m**, **10n**, **10r** and **10s** are the most potent in the series. All of these active compounds contain a substituted phenyl ring attached to the pyrimidine unit (R^2). Remaining molecules displayed either moderate or weak inhibition activity. The active molecules depict a trend wherein halogen substitutions mainly consist of fluoro or chloro groups at either position-3 or 4 of the phenyl ring. On the other hand, bromo, methoxy and methyl groups did not impart much enhancement in the activity. Furthermore, an overall comparison between compounds **10a–10k** and **10l–10v** hints at slightly improved activity of the latter set which may be due to the presence of bioisostere fluoro group on the indole core (R^1). It can also be seen that the potency of the active molecules is comparable with that of the standard drugs (Table 3).

5. Conclusions

The paper describes the synthesis of a new library of indole-3-pyrimidine hybrids consisting of an amide linkage at position-2 of the indole ring and a diversely substituted phenyl ring on the pyrimidine moiety. The structural identification of all the intermediates and target compounds was carried out by spectroscopic techniques namely ^{1}H NMR, ^{13}C NMR, ESI–MS

and elemental analyses. The target molecules were investigated for their *in vitro* anti-proliferative and antimicrobial properties by MTT assay and disc diffusion methods respectively. The most susceptible cell lines comprised of HepG2 and MCF-7 with the majority of compounds showing higher inhibition against these cell lines. Compounds **10f**, **10o** and **10v** inhibit significantly the growth of all three cancerous cells indicating their broad spectrum anti-tumour activity. On the other hand, in the case of anti-microbial studies, compounds **10b**, **10c**, **10g**, **10h**, **10m**, **10n**, **10r** and **10s** which contain a chloro or fluoro substitution (R^2) at either position-3 or 4 of the phenyl ring displayed higher potency than the rest of the molecules. Moreover, low toxicity on the benign Vero cells and higher efficacy of the active molecules may provide a potential lead for the development of novel therapeutic agents in future.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jscs.2015.09.003.

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